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Effect of Cortisone upon the Tissue Synthesis of Acid Mucopolysaccharide

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In earlier papers (1, 2, 3), we have demonstrated the anabolic utilization of sulfate ion by animal tissues. This utilization is especially marked in embryonic tissues, tissues from new-born animals, and the regenerating tissue from healing muscle wounds in adults. It is also significant in the vascular tissue of young adult animals.

The figure will indicate the relative sulfate affinities at different stages of embryonic development of the chick.

The first table will give an idea of the relative tissue affinities, and the quantities of chondroitin sulfate or «sulfomucopolysaccharide» synthesized during the time indicated.

In table 1 are shown values for chondroitin sulfate synthesis by wound tissues maintained *in vitro*. Especially to be noted is the striking similarity between the sulfate metabolism of granulation tissue and of embryonic tissue at its stage of maximum sulfate affinity (see fig. 1).

We believed that the high affinity of aortic tissue for sulfate may be quite significant in the pathological calcification of this tissue. It was thought also that an abnormal rate of mucoprotein or mucoid synthesis might be involved in the development of tissue lesions in several other diseases. These include the mesenchymal diseases associated with increased metachromasia (i.e. rheumatic heart disease, arthritis, etc.),

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Table 1

Synthesis of chondroitin sulfate by chick tissues[1] in vitro

Tissue	No. Chicks (N)	No. Samples	μg chondroitin sulfate synthesized per 100 mg wet tissue $\pm$ average error
Lateral Condyle of tibia	6	12	45. $\pm$1.0
Aortic Arch	6	12	13. $\pm$0.6
Spleen	6	18	6. $\pm$0.4
Kidney	6	18	5. $\pm$0.5
Shaft of tibia	6	18	2.7 $\pm$0.1
Red Marrow of tibia	6	24	1.7 $\pm$0.1
Heart ventricle	6	24	0.85 $\pm$0.2
Skeletal muscle	6	24	0.3 $\pm$0.1
Tissue from healing muscle wound[2]	10	30	5.4 $\pm$1.0

[1] Normal chick tissues cultured on the 6th day after hatching, 3.0–3.5 mg samples of tissue cultured 45 hours at 37° C. in Tyrodes solution containing 4.8 mg/liter sulfate ion labeled with radioactive sulfur, S^{35}.

[2] Wound tissues from sterile healing wounds on fifth day after partial section of M. pectoralis major in chickens two months of age.

Buerger's disease, amyloid nephroses, mucoid colitis, and the peculiar exophthalmos elicited by excess pituitary thyrotropin.

After the appearance of our papers, our attention was called to the work of *Ragan* and his associates (4) upon the inhibitory effect of cortisone upon wound granulation. It appeared to us that cortisone might exert its palliative action in the mesenchymal diseases through the same mechanisms as are involved in wound healing.

Studies made upon the rate of synthesis of chondroitin sulfate in healing and non-healing wounds in young chickens indicated that chondroitin sulfate was not being synthesized (nor was metachromasia apparent) in the non-healing wounds of chickens treated with high dosages of cortisone acetate (35–45 mg/kg body-weight). The ratios of newly synthesized labeled chondroitin sulfate synthesized in muscle wounds as compared to neighbouring unwounded muscle are shown for cortisone-treated chickens as compared to controls, in table 2.

It will be noted that, in the controls, wound tissue showed four times as much activity as was found for the neighbouring unwounded tissue. In the cortisone treated animals the wound tissues showed no increase in chondroitin sulfate.

Because of the fact that quantitative values can be more easily obtained when tissues are studied *in vitro*, it was decided that our quantitative method (1, 2) should be used.

Table 2

Ratio of specific activities of wound tissue to unwounded tissue of the same animal (Chickens injected with carrier-free $Na_2S^{35}O_4$ and sacrificed five days later)

Group	Ratio of specific activity of tissues as $BaS^{35}O_4$ in 30 mg $BaSO_4$ carrier wound tissue/normal tissue
Controls.	3.5/1
Cortisone treated (35 mg/ day/kg body weight) . .	1/1

Surviving tissues from rapidly growing embryos and from healing and non-healing wounds were maintained in a medium containing labeled inorganic sulfate and graded concentrations of cortisone (free alcohol). It was found that graded levels of cortisone effected graded inhibition upon the synthesis of chondroitin sulfate or «sulfomucopolysaccharide» (if we may coin a more general term). Some of our data for wound tissue are shown in table 3.

Table 3

Effect of cortisone alcohol in vitro upon the synthesis of chondroitin sulfate and soluble sulfate esters by tissue from healing wounds in chickens[1]

Concentration of cortisone in medium mg/liter	Sulfate fixed calculated as chondroitin sulfate μg/100 mg tissue	Weight of sulfate ion esterified μg/100 mg tissue (soluble ester sulfate)
No cortisone (controls)	6.6	0.7
25 mg/l	5.6	0.6
50 mg/l	2.4	0.4
75 mg/l	1.4	0.2
120 mg/l	0.4	0.1

[1] Tissues removed on fifth day after wounding.– Tissues cultured 45 hours at 37° C in Tyrode solution containing 4.8 mg/liter of sulfate ion labeled with 6×10^7 counts radioactive sulfur per mg sulfate ion.

Similar concentrations of cortisone had no apparent effect during 60 hours, upon the migration of fibroblasts or the pulsation of explants of heart tissue cultured in Carrel flasks. Desoxycorticosterone alcohol caused early degeneration of fibroblasts, and appeared to cause death of the tissues *in vitro*. Pulsation of heart tissue and migration of fibroblasts ceased after the addition of desoxycorticosterone. It appeared to us that cortisone inhibited the synthesis of «sulfomucopolysaccharide» or chondroitin sulfate without greatly affecting those activities essential

to survival of tissues *in vitro*. We have not yet studied the effect of cortisone upon the protein metabolism of tissues *in vitro*. It would appear from our own studies on rats, and those of other workers upon human patients and animals, that the palliative action of cortisone in the collagen diseases does not depend upon its antianabolic or procatabolic effect in protein metabolism. We have found that compound A acetate has an effect identical with that of cortisone upon protein metabolism in the intact rat, yet it is said to have no therapeutic value in collagen disease. Starvation leads to a negative nitrogen balance, but has only moderate influence upon the mesenchymal diseases.

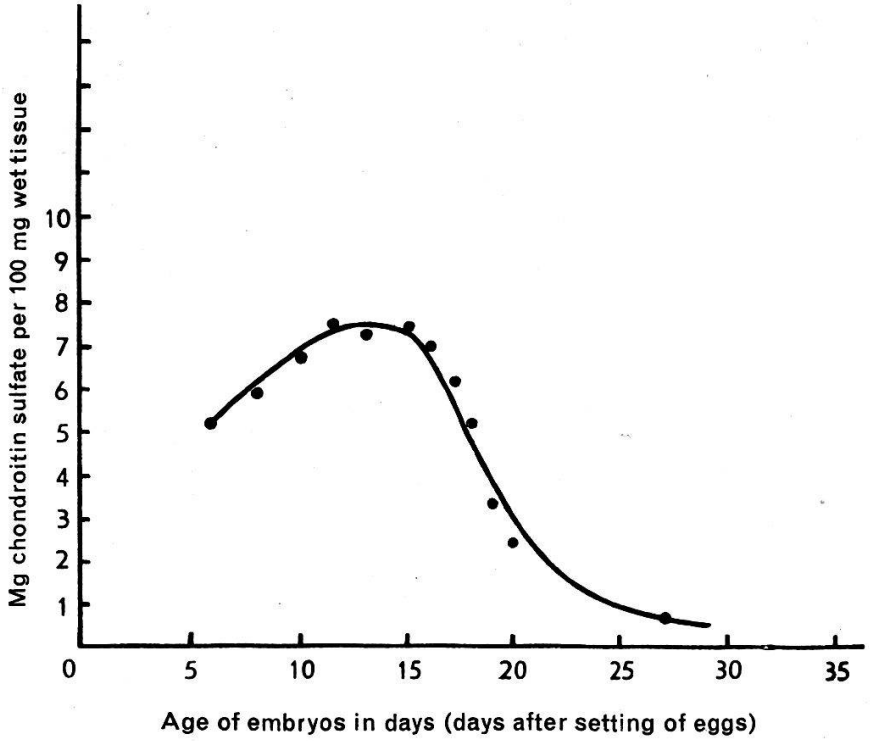

Fig. 1. Synthesis of chondroitin sulfate by heart tissue *in vitro*.

Since scurvy is associated with decreased activity in the connective tissue, failure to form normal granulation tissue, and decreased metachromasia, it would be interesting to study the effect of mild scurvy upon the course of rheumatoid arthritis.

In one preliminary experiment involving only three guinea pigs, it was found that the skin of the scorbutic (moribund) pig had a fixed sulfate concentration only one half as great as that of the normal pig. The cortisone-treated pig (30 mg cortisone acetate per day and per kg body-weight) had a fixed sulfate content equivalent to three fourths that of the control (it is realized that no significance can be claimed for results obtained upon only one experimental animal). It is possible

however, that a deficiency of vitamin C or citrin might partly alleviate or modify the development of tissue lesions by decreasing the fibroblastic activity as has been observed in the delayed healing of wounds in scorbutic animals and man.

Summary

It may be that the primary effect of cortisone is in the abolition of the inflammatory reaction of the tissue. Our results with tissues from healing wounds, where the inflammatory reaction has already been initiated, and with rapidly growing embryonic tissue, where the stimulatory mechanism is operating, would appear to indicate an additional, direct, inhibitory action of cortisone upon the reactivity of those connective tissue cells responsible for the formation of the ground substance and the granulation tissue of healing wounds. It is suggested that the direct action of cortisone may be partly responsible for its palliative action in certain connective tissue diseases.

1. *Layton, L. L.:* Cancer **3,** 725 (1950). – 2. *Layton, L. L.:* Proc. Soc. exper. Biol. a. Med. (Am.) **73,** 570 (1950). – 3. *Layton, L. L.:* Proc. Soc. exper. Biol. a. Med. **76,** 596 (1951). – 4. *Ragan, Ch.* et al.: Proc. Soc. exper. Biol. a. Med. (Am.) **72,** 718 (1949).